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Does vitreous galactin-3, copeptin and retina binding protein-4 concentrations change in diabetic retinopathy?

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ABSTRACT

Aims: This study aimed to investigate whether the concentrations of Galactin-3 (G-3), Copeptin (CP) and Retina Binding Protein-4 (RBP-4) are affected in the vitreous humor of patients with diabetic retinopathy (DR).

Methods: Thirty-six patients with diabetes mellitus (DM) were included in the study, consisting of 10 patients without DR and 26 patients with proliferative diabetic retinopathy (PDR). The control group comprised 15 patients who underwent vitrectomy for epiretinal membrane and macular hole surgeries. Vitreous CP, G-3, and RBP-4 concentrations were examined using the enzyme-linked immunosorbent assay (ELISA) method. The groups were compared internally

Results: We did not observe any significant differences in the concentrations of G-3, CP and RBP-4 in the vitreous humor between diabetic patients and the control group (p=0.56, p=0.65 and p=0.11, respectively). When comparing vitreous samples of diabetic subgroups with and without DR findings to the control group, no significant differences were detected (p=0.51, p=0.66, and p=0.19, respectively).

Conclusion: Our results indicate that the concentrations of G-3, CP, and RBP-4 in the vitreous humor remain unchanged in both diabetic patients and those with proliferative diabetic retinopathy (DRP).

Keywords: Copeptin, galactin-3, retina binding protein-4, diabetes mellitus, diabetic retinopathy

INTRODUCTION

Diabetic retinopathy (DR) is one of the most severe chronic microvascular complications of diabetes mellitus (DM) and ranks among the leading causes of vision impairment and irreversible blindness in adults in developed countries.[1] Considering the increasing prevalence of diabetes, longer life expectancies, and the aging population, it is estimated that the number of DR patients could reach up to 191 million by the year 2030. This poses a significant economic burden both on individuals and society as a whole.[2]

Among the most important risk factors in the development of DR are considered to be the duration of diabetes and glycemic control, while the impact of dyslipidemia, hypertension, and obesity on DR has also been demonstrated.[3] It has been shown that regulating serum glucose, hemoglobin A1c, and lipid levels is essential for controlling DR in diabetic patients and reducing the severity of DR progression.[4] However, achieving normoglycemia has not always proven to be effective in preventing DR progression, suggesting that other additional factors may play a role in the development of DR.

One of the key factors in the pathogenesis of DR is chronic low-grade inflammation triggered by glycolytic metabolites. Numerous studies have been conducted on the significant role of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), IL-8, and IL-1β in DR inflammation. These studies have found a substantial correlation between proinflammatory markers and the severity of DR.[5] However, the exact pathogenesis of DR remains not fully understood, and much remains unknown.

Galactin-3 (G-3) is a protein belonging to the lectin family, which binds to galactose. It plays a role in many biological processes such as apoptosis, cellular growth, differentiation, proliferation, cellular adhesion, and tissue preservation.[6] G-3 can promote the secretion

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of other proinflammatory factors such as TNF-α and IL-6 by activating macrophages in a dose-dependent manner.[7] It serves as a potent inflammatory promoter, contributing to the initiation of the inflammatory response associated with acute and chronic inflammation by facilitating chemotaxis in monocytes and macrophages.[8]

Copeptin (CP) represents the C-terminal portion of the precursor of arginine vasopressin (AVP). CP is a reliable and clinically useful biomarker that can be used as a substitute for AVP. Somatic stress plays a significant role in the regulation of CP. CP is recognized as a prognostic marker in various acute diseases, including sepsis, myocardial infarction, pneumonia, or ischemic stroke.[9,10] Due to its positive correlation with disease severity, CP is considered a prognostic factor.

Retinal binding protein-4 (RBP4) is a newly identified adipokine primarily associated with retinol and secreted by white adipose tissue.[11] It can induce CD4 T cell Th1 polarization and trigger inflammation in adipose tissue by activating antigen-presenting cells.

In our study, we aimed to determine the relationship between DM and DR of G-3, CP, and RBP-4, which are associated with inflammation.

METHODS

The study was carried out with the permission of Tokat Gaziosmanpaşa University Clinical Researches Ethics Committee (Date: 02.09.2021, No: 21KAEK-195). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This prospective and randomized study was conducted between January 2022 and May 2023. All patients gave written informed consent before their participation.

The study included thirty-six patients who were diagnosed with Diabetes Mellitus (DM) and followed up with. The control group comprised 15 individuals who had undergone vitrectomy surgery for conditions like epiretinal membrane, macular hole, or retinal detachment. DM patients were divided into two subgroups according to the International Classification of Clinical Diabetes Retinopathy as proliferative DR (PDR) and without DR findings.

The patients preoperative glucose, HbA1c, low density lipoprotein (LDL), and triglyceride values were recorded.

Patients with a history of previous eye diseases such as glaucoma, corneal neovascularization, and uveitis, as well as those who had previously undergone vitrectomy surgery, were excluded from the study.

Vitreous Samples Collection

Undiluted vitreous fluids were collected from patients before undergoing primary pars plana vitrectomy. Vitreous samples were obtained using a three-port 25-gauge transconjunctival suture-less vitrectomy system and were directly suctioned into a 5 ml syringe. The vitreous samples were stored at -80°C for six months for subsequent analysis, ensuring prevention of repeated freeze-thaw cycles.

The concentrations of Vitreous G-3 were measured using a commercially available Human Galectin-3 Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Sun-Red Bio Company, Catalog No. 201-12-1952, Shanghai, China). Enzymatic reactions were measured in an automated microplate photometer (BioTek Instruments Inc. Synergy 4, Serial No. 233513, USA). G-3 concentrations were determined by comparing the optical density of the samples to a standard curve. The inter-assay and intra-assay coefficients of variation for G-3 were <12% and <10%, respectively. The kit's test range was 0.2-60 ng/ml, and the sensitivity of the test was 0.186 ng/ml. All analyses were performed according to the manufacturer's instructions.

The concentrations of Vitreous CP were measured using a commercially available Human CP ELISA Kit (Sun-Red Bio Company, Catalog No. 201-12-5463, Shanghai, China). Enzymatic reactions were measured in an automated microplate photometer. CP concentrations were determined by comparing the optical density of the samples to a standard curve. The inter-assay and intra-assay coefficients of variation for CP were <12% and <10%, respectively. The kit's test range was 0.07-20 ng/ml, and the sensitivity of the test was 0.067 ng/ml. All analyses were performed according to the manufacturer's instructions.

The concentrations of Vitreous RBP-4 were measured using a commercially available Human RBP-4 ELISA Kit (Sun-Red Bio Company, Catalog No. 201-12-1207, Shanghai, China). Enzymatic reactions were measured in an automated microplate photometer. RBP-4 concentrations were determined by comparing the optical density of the samples to a standard curve. The inter-assay and intra-assay coefficients of variation for RBP-4 were <12% and <10%, respectively. The kit's test range was 0.6-180 mg/L, and the sensitivity of the test was 0.518 mg/L. All analyses were performed according to the manufacturer's instructions.

Statistical Analysis

The statistical analysis was performed using SPSS 22.0 software. To assess the normal distribution of variables, visual and analytical methods such as the Kolmogorov-Smirnov and Shapiro-Wilk tests were employed.

Descriptive analyses were presented using means and standard deviations for variables that showed a normal distribution. To compare differences in characteristics between different groups, the chi-square test and Mann-Whitney U-test and Kruskal-Wallis test was used. P value less than 0.05 was defined statistically significant.

RESULTS

In this study, vitreous samples were analyzed from a total of 51 patients, including 36 diagnosed with DM and 15 in the control group. Among the DM patients, 10 showed no evidence of DR, while 26 were diagnosed with proliferative DR. While there was a significant difference in glucose levels between the control and DM groups, there were no significant differences in LDL and triglyceride levels (p=0.024, p=0.94, p=0.85, respectively). When comparing the DR subgroups, although glucose, HbA1c, LDL, and triglyceride levels were generally higher in the proliferative DR group, the differences were not statistically significant (p=0.3, p=0.13, p=0.66, and p=0.09, respectively). The demographic characteristics and laboratory values of the groups are presented in **Table 1**.

There was no significant difference in G-3, CP and RBP-4 concentrations between individuals without DM and those with DM (**Table 1**). In addition, there was no significant difference between the control group and DR subgroups (p=0.51, p=0.66, and p=0.19, respectively).

DISCUSSION

The pathogenesis of DR involves metabolic pathways such as the polyol pathway, advanced glycation end products pathway, hexosamine pathway, and protein kinase C pathway. With the increase in blood glucose levels, these metabolic pathways become activated. Oxidative balance is disrupted, leading to oxidative stress. This, in turn, activates apoptosis in mitochondria and results in neurovascular dysfunction. Additionally, oxidative stress leads to increased inflammation through cytokine upregulation, resulting in hypoxia and an increase in vascular endothelial growth factor.[12] Despite numerous studies on the pathogenesis and treatment of DR, it remains incompletely understood.

The current study was conducted to evaluate the vitreous humor concentrations of G-3, CP, and RBP-4, which are considered proinflammatory biomarkers, and their relationship with DM and DR.

While there have been various studies investigating the association of G-3, CP and RBP-4 with DR development and progression, all of them focused on evaluating serum concentrations. No prior study has examined the relationship between vitreous fluid concentrations of these biomarkers and DR. Thus, we decided to investigate this association.

G-3 is known as a proinflammatory molecule that triggers the inflammatory response and oxidative stress.8 Existing literature has linked serum G-3 concentrations to various diseases, including gastritis, asthma, cancer, heart diseases, kidney diseases, and obesity.[6] G-3's stability as a biomarker, its independence from factors like gender, age, and body mass index, and its lack of circadian variation increase its applicability in disease diagnosis and prognosis.[13,14]

The effect of G-3 on chronic inflammation in DM is not fully understood. Some studies have suggested that G-3 delays the development of diabetes by preventing the chronicization of the inflammatory process, while others indicate that it worsens diabetes progression by increasing inflammation and fibrosis.[6] Experimental studies on mice lacking G-3 showed increased fat accumulation and insulin resistance along with increased inflammation in adipose tissue.[15] Another study found that G-3-deficient mice exhibited higher hyperglycemia and impaired glucose tolerance compared to control wild-type mice, suggesting that G-3 deficiency contributes to diabetes pathogenesis. These studies have argued that G-3 plays a protective role against both obesity and DM, regulating natural susceptibility to overnutrition.[16,17]

Table 1. Basal characteristic of controls and diabetes patients with DR or without DR.

	Control n: 15	Diabetes mellitus n: 36	Retinopathy status		p
			No (n: 10)	Yes (n: 26)	
Age	68.5±7.4	64.3±9.1	71.1±9.3	61.7±7.8	0.12
Male (%)	10 (66.6%)	17 (47.2%)	5 (50%)	12 (46.1%)	0.24
Glucose (mg/dl)	95.5±30	187.7±73[a]	162.5±48	194.9±78	0.015
HbA1c (%)	-	9.44±2.1	9.07±1.9	10.6±2.5	0.13
LDL (mg/dl)	138.6±16.5	140.5±5.6	130.4±16.8	143.1±17.5	0,94
Triglyceride (mg/dl)	226.6 ±93	235.8±130	230.8±124	241.2±156	0.85
Galactine-3 (ng/ml)	10.36±3.0	10.48±3.1	10.88±2.7	10.25±3.1	0.52
Copeptin (ng/ml)	9.65 ±1.67	9.78±2.4	9.84±2.02	9.76±2.64	0.65
Retina binding protein-4 (ng/ml)	81.6±18.1	75.17±22.6	75.29±11.4	75.12±25.8	0.11

LDL=Low Density Lipoprotein, p Mann-Whitney U test compared control and diabetes mellitus group

In a study by Mendonça et al.[18] G-3 deficiency in mice led to reduced inflammation and preserved neuronal, retinal, and optic nerve structures after 8 weeks of diabetes. Higher circulating G-3 levels in DM patients have been associated with microvascular and macrovascular complications.[19]

CP inhibits Na^+-K^+-ATPase activity by binding to receptors on the cell membrane, disrupting vascular functions by inhibiting nitric oxide synthesis. It has been argued that CP participates in the retinal vascular endothelial function and contributes to the DR process.[20] Zhu et al.[21] found that increased CP levels were associated with DR and diabetic nephropathy independently of DM in their study involving 306 patients, suggesting a potential role for CP in the pathophysiology of DM. Additionally, they found correlations between plasma copeptin levels and the severity of insulin resistance and disease duration.

In a study by Li et al.[22] CP levels were found to be higher in DM patients compared to the healthy group, and when comparing DR within the DM group, PDR exhibited higher CP concentrations than nonproliferative DR (NPDR). CP demonstrated different increasing trends as DR clinical stages progressed, suggesting that CP might be a determining factor for DR.

RBP4 is a newly discovered adipocyte-secreted hormone associated with obesity and known to play a role in insulin resistance.[23] Akbay et al.[24] argued that there was no association between RBP-4 and DR in DM patients, but they proposed that serum RBP-4 levels could be influenced by kidney functions. However, Malechka et al.[25] suggested that RBP-4 plays a significant role in DR incidence and progression.

The connection between increased serum RBP-4 levels and DR incidence has been speculated to be related to increased inflammation in human retinal microvascular endothelial cells.[26] Du et al.[27] argued that RBP-4 contributes to DR progression through the upregulation of retinal IL-18 protein expression, leading to proinflammatory mechanisms. Studies with mice treated with RBP4 antagonists reported reduced vascular leakage.[28] Zhang et al.[29] reported that dietary intervention with retinol significantly reduced RBP-4 levels and DR incidence. Sun et al.[30] found higher RBP-4 levels in DR patients with type 1 DM in their study.

Contrary to previous studies, our current study did not find any significant difference in G-3, CP, and RBP-4 levels in vitreous humor between DM and DR patients and the control group. To the best of our knowledge, this study is the first to compare vitreous fluid concentrations of these biomarkers in DM, and thus, no previous study has been conducted on this specific aspect. Previous investigations were limited to either animal experiments or studies involving serum samples. This study, therefore, is the first to evaluate the proportions of these molecules in vitreous humor in the context of DM. It is a well-established fact that molecules can have different functions in various regions of the body and, as a result, may be expressed at different levels in different tissues, such as serum and vitreous. In our other study, there was a difference in serum samples, and the reason why there was no difference in the vitreous sample can be attributed to this.

There are some limitations to our study. First, the sample size was relatively small. Additionally, patients in the control group had other accompanying diseases. Another shortcoming of the study is that serum samples cannot be analyzed and compared with previous studies and vitreous samples. Therefore, further studies with larger sample sizes and more comprehensive control groups are needed for confirmation.

CONCLUSION

Our study found no significant differences in G-3, CP, and RBP-4 vitreous concentrations between DM and DR patients and the control group.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University Clinical Researches Ethics Committee (Date: 02.09.2021, No: 21KAEK-195).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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